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Generation of Surface-bound Multicomponent Protein Gradients

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In biological systems, gradients of soluble or matrix-bound stimuli are crucial to cellular processes such as directed cell migation, axon guidance and embryonic development[1–4]. In engineered systems, spatially controlled presentation of molecular cues enables the study of gradient-sensing mechanisms *in vitro* and the development of synthetic microenvironments that elicit programmed cellular responses. Here we describe a new approach to the generation of surface-bound multicomponent protein gradients.

Controlled concentration gradients of molecules in solution have been realized in microfluidic channels,[5] and nonspecific adsorption and covalent crosslinking have been used to immobilize protein gradients on solid surfaces.[6–9] The method described here requires neither crosslinking nor nonspecific adsorption of the protein of interest. Instead we use a modular design in which immobilization is mediated by engineered coiled-coil domains that are easily appended to essentially any protein.

We recently described artificial polypeptide scaffolds that enable convenient preparation of protein microarrays through strong association of the ZE/ZR heterodimeric leucine zipper pair.[10] Here we show that integration of such scaffolds into microfluidic systems provides a simple route to multicomponent protein gradients. Two proteins, a mutant tenth fibronectin type III domain[11] (FN), and a *de novo* designed vascular endothelial growth factor mimetic peptide[12] (QK), were selected as model species for gradient generation. Each was fused at its C-terminus to the acidic leucine zipper peptide ZE to yield fusion proteins FNZE and QKZE, respectively (see supporting information for amino acid sequences). The scaffold (designated ZRELF) was a multidomain artificial protein comprising an N-terminal basic leucine zipper peptide (ZR) fused to the previously described elastin-mimetic domain ELF. Bacterial expression of ZRELF in medium supplemented with the photoreactive amino acid *p*-azidophenylalanine (pAzF)[10] yields a photocrosslinkable substrate for controlled microfluidic deposition of proteins fused to ZE (Figure 1).

Glass slides pre-treated with octyltrichlorosilane were spin-coated with a solution of 2.5 mg/ml ZRELF dissolved in 50% 1-propanol (the organic solvent helped wet the hydrophobic surface). The resulting protein films were crosslinked to the substrates through photodecomposition of pAzF by 2 minute exposure to a mercury arc lamp. A microfluidic

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gradient generator (Figure 1C) fabricated from poly(dimethylsiloxane) (PDMS) was sealed to the crosslinked ZRELF film. Solutions containing ZE-tagged proteins were continuously pumped through the microfluidic network by a syringe pump. The fluid streams introduced through the two inlets were combined and mixed in the serpentine channels to establish a concentration gradient across the output channel. Protein gradients were deposited on the ZRELF film via coiled-coil association between ZE and ZR. After generation of surface-bound gradients, the device was soaked in water and the PDMS portion above the gradient region was gently removed. Figure 1D shows a typical fluorescence image representing an immobilized counter-gradient of QKZE and FNZE across the output channel (800 μm wide and 10 mm long).

The ZE peptide was used as a density controller to adjust the number of binding sites available for protein immobilization. For example, we generated a gradient of FNZE by injecting ZE and FNZE (each at a concentration of 100 nM) through the two inlets. The corresponding density profile of FNZE was obtained (right panel of Figure 2A) from a calibrated fluorescence image of the gradient (left panel of Figure 2A),. The density of FNZE varied from zero to 4.5 molecules/100 nm^2 across the 800 μm channel with a slope of 5.6×10^{-8} molecules/nm^3.

To investigate the possibility of generating gradients containing multiple species, a mixture of QKZE and FNZE was introduced through one inlet, while a solution of ZE was introduced through the other. The concentration of each solution was 100 nM. As shown in Figure 2B, parallel gradients of FNZE and QKZE were created. The density of FNZE increased from zero to 1.8 molecules/100 nm^2 across the channel, while that of QKZE increased from zero to 6.4 molecules/100 nm^2. Although FNZE and QKZE were introduced at equal concentrations, the resulting surface densities were significantly different. Perhaps the small size of QKZE (~9 kD) relative to FNZE (~17 kD) allows faster binding to ZRELF. Reducing the concentration of QKZE to 50 nM yielded gradients in which the protein densities were more closely matched (Figure 2C). Counter-gradients were readily generated by delivering QKZE and FNZE through opposing inlets (Figure 2D).

Human umbilical vein endothelial cells (HUVECs) respond to the local density of FN domains on gradient surfaces. On a control substrate carrying only ZE peptide, very few HUVECs attached, and those that were retained by the surface did not spread (Figure 3A). In contrast, significant cell adhesion was observed on FNZE surfaces (Figure 3B), demonstrating that cells recognize the adhesion ligand presented by the recombinant protein. The attached cells were well spread and distributed homogeneously across the substrate.

HUVECs seeded on the gradient shown in Figure 2A did not distribute uniformly (Figure 3C); the number of adherent cells increased with the density of cell adhesion ligands. To quantify the extent of cell attachment, each field of view (800 μm × 600 μm) was divided into three regions, and the fraction of adherent cells was determined by dividing the number of cells per region by the total number of cells attached (Figure 3D). The fraction of attachment increased from 0.13 ± 0.05 at low FNZE density to 0.51 ± 0.06 at high density.

Integration of microfluidics, protein engineering and surface functionalization provides a simple and flexible approach to the generation of surface-bound multicomponent protein gradients. The method can be applied to essentially any combination of proteins or peptides. Protein densities and gradient shapes are easily and precisely controlled. Because it is straightforward to engineer the affinities of leucine zipper pairs[14], it should be possible to construct dynamic surfaces that allow spatiotemporal control of the presentation of protein ligands. Finally, incorporation of leucine-zipper pairs into protein hydrogels[15] may enable extension of the method to the generation of gradients in three-dimensional matrices.

Acknowledgments

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References

1. Christiansen JH, Goles EG, Wilkinson DG. Curr Opin Cell Biol. 2000; 12:719. [PubMed: 11063938]
2. Dickson BJ. Science. 2002; 298:1959. [PubMed: 12471249]
3. Carmeliet PMJ. Nat med. 2000; 6:389. [PubMed: 10742145]
4. Ashe HL, Briscoe J. Development. 2006; 133:385. [PubMed: 16410409]
5. Jeon NL, Dertinger SKW, Chiu DT, Choi IS, Stroock AD, Whitesides GM. Langmuir. 2000; 16:8311.
6. Dertinger SK, Jiang X, Li Z, Murthy VN, Whitesides GM. Proc Natl Acad Sci USA. 2002; 99:12542. [PubMed: 12237407]
7. Petty RT, Li HW, Maduram JH, Ismagilov R, Mrksich M. J Am Chem Soc. 2007; 129:8966. [PubMed: 17602634]
8. Gunawan RC, Choban ER, Conour JE, Silvestre J, Schook LB, Gaskins HR, Leckband DE, Kenis PJ. Langmuir. 2005; 21:3061. [PubMed: 15779985]
9. For excellent recent reviews of preparation and applications of gradients in soft materials, see Genzer J, Bhat RR. Langmuir. 2008; 24:2294. [PubMed: 18220435] Kim MS, Khang G, Lee HB. Prog Polym Sci. 2008; 33:138.
10. Zhang KC, Diehl MR, Tirrell DA. J Am Chem Soc. 2005; 127:10136. [PubMed: 16028902]
11. Richards J, Miller M, Abend J, Koide A, Koide S, Dewhurst S. J Mol Biol. 2003; 326:1475. [PubMed: 12595259]
12. D'Andrea LD, Iaccarino G, Fattorusso R, Sorriento D, Carannante C, Capasso D, Trimarco B, Pedone C. Proc Natl Acad Sci USA. 2005; 102:14215. [PubMed: 16186493]
13. Hoover DM, Lubkowski J. Nucleic Acids Res. 2002; 30:e43. [PubMed: 12000848]
14. Moll JR, Ruvinov SB, Pastan I, Vinson C. Protein Sci. 2001; 10:649. [PubMed: 11344333]
15. Shen W, Zhang K, Kornfield JA, Tirrell DA. Nat Mater. 2006; 5:153. [PubMed: 16444261]

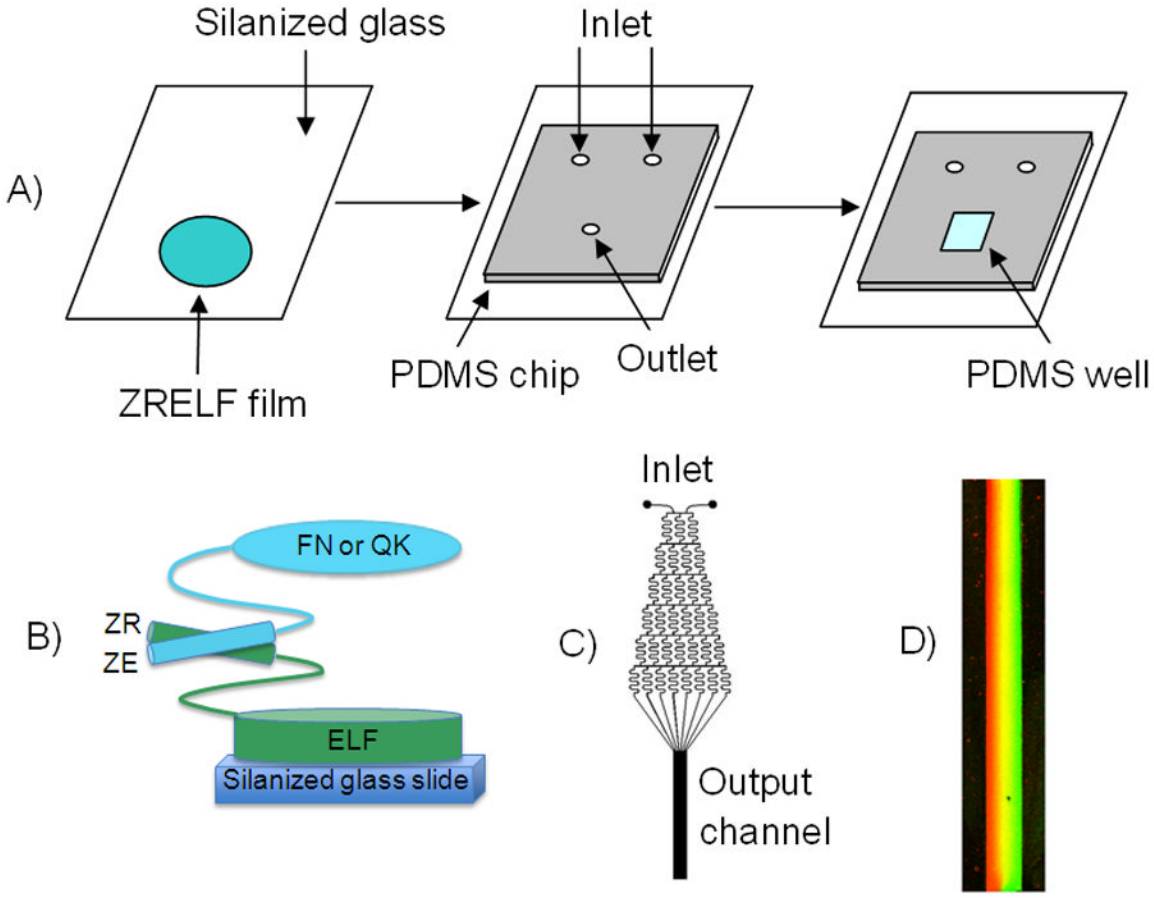

Figure 1.

A) Assembly of the microfluidic device. B) Protein immobilization via leucine zipper dimerization. C) Schematic design of the gradient generator. D) Fluorescence image of a counter-gradient of QKZE (labeled with Cy3) and FNZE (labeled with Alexa 647) across the output channel. The channel is 800 μm wide and 10 mm long.

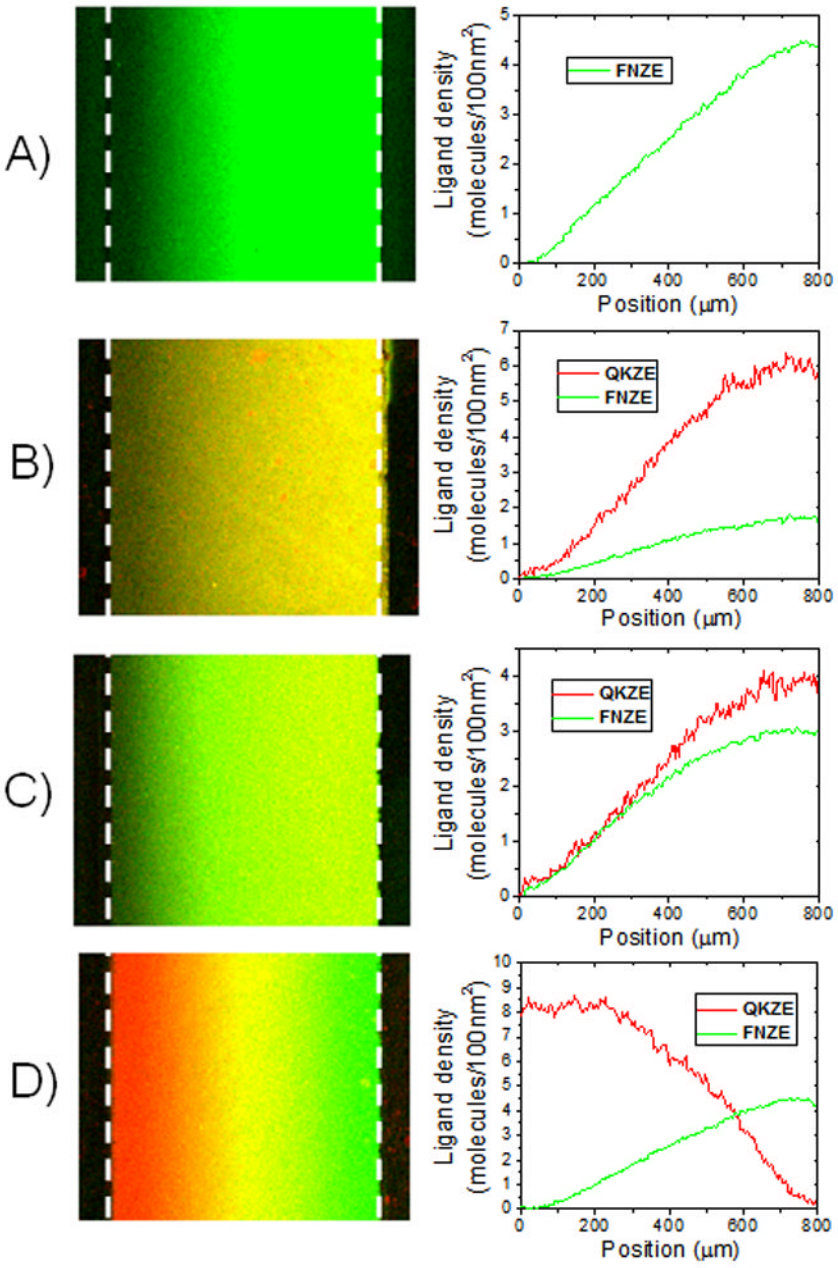

Figure 2.

Fluorescence images of immobilized gradients and their corresponding protein density profiles. FNZE was labeled with Cy3; QKZE was labeled with Alexa 647. Cy3 was pseudocolored green, Alexa 647 red and overlay yellow. A) Left inlet ZE (100 nM); right inlet FNZE (100 nM); B) Left inlet ZE (100 nM); right inlet FNZE (100 nM) plus QKZE (100 nM); C) Left inlet ZE (100 nM); right inlet FNZE (100 nM) plus QKZE (50 nM); D) Left inlet QKZE (100 nM); right inlet FNZE (100 nM).

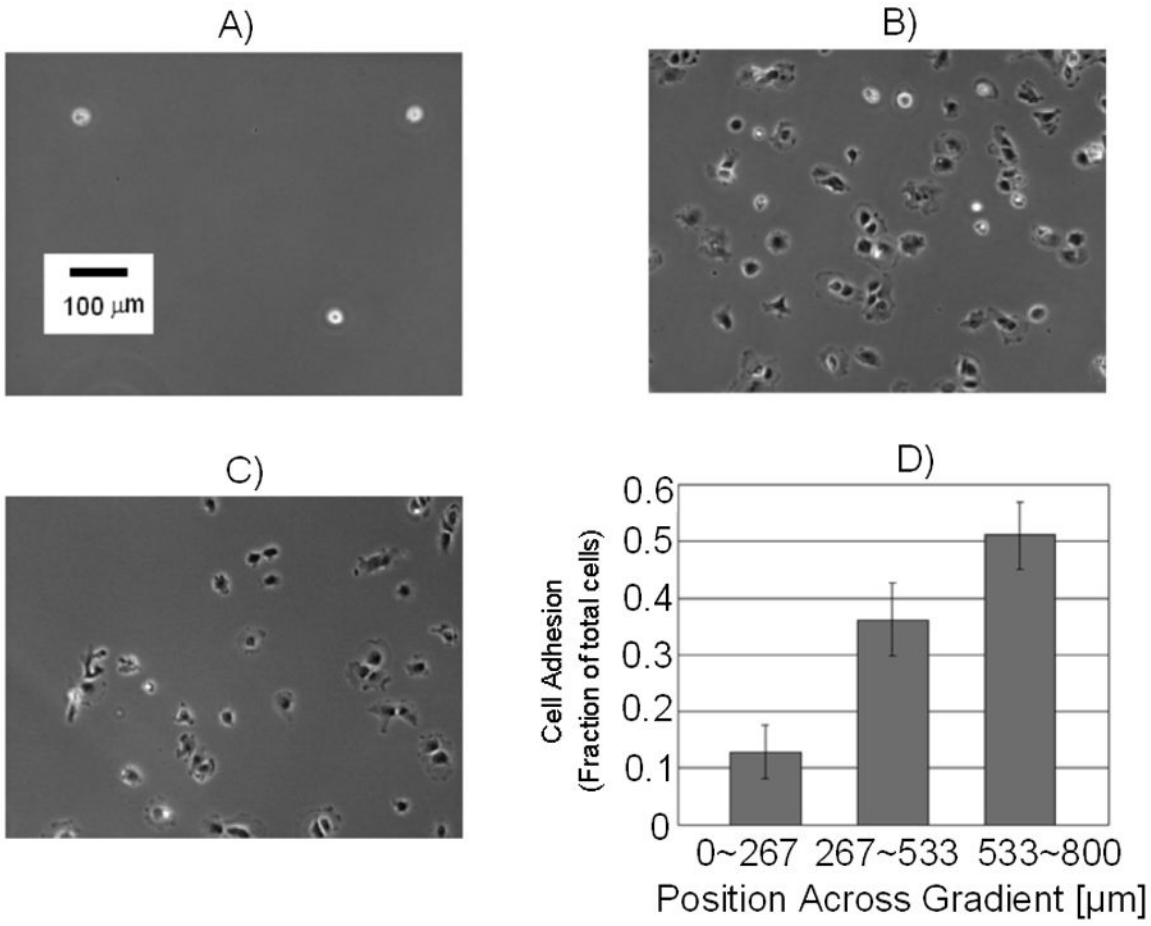

Figure 3.
Attachment of HUVECs to uniform and gradient surfaces. Phase contrast images of HUVECs attached to the surface of A) ZE, B) FNZE, C) FNZE gradient shown in Figure 2A. D) Variation in extent of HUVEC adhesion as a function of position on FNZE.